Synthesis of a Tetronic Acid Library Focused on Inhibitors of Tyrosine and Dual-Specificity Protein Phosphatases and Its Evaluation Regarding VHR and Cdc25B Inhibition

Mikiko Sodeoka,*[,†,‡] Ruriko Sampe,[‡] Sachiko Kojima,[‡] Yoshiyasu Baba,[†,‡] Takeo Usui,[§] Kazunori Ueda,[§] and Hiroyuki Osada*[,§]

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Katahira, Aoba, Sendai 980-8577, Japan, Sagami Chemical Research Center, Nishi-Ohnuma, Sagamihara 229-0012, Japan, and RIKEN, Hirosawa, Wako-shi 351-0198, Japan

Received October 2, 2000

Selective inhibitors of protein tyrosine phosphatases (PTPs) and dual-specificity phosphatases (DSPs) are expected to be useful tools for clarifying the biological functions of the PTPs themselves and also to be candidates for novel therapeutics. We planned a library approach for the identification of PTP/DSP inhibitors in which 3-acyltetronic acid is used as a "core" phosphate mimic. A series of novel tetronic acid derivatives were synthesized and evaluated as inhibitors of the dual-specificity protein phosphatases VHR and cdc25B. Several compounds are found to be potent inhibitors of cdc25B, which is a key enzyme for cell-cycle progression. The promising results described herein strongly indicated that this tetronic acid library is potent as a library focused on the PTP/DSP-selective inhibitor.

Introduction

Tyrosine phosphorylation of proteins is a fundamental mechanism of intracellular signal transduction involved in important cellular events such as cell growth and differentiation.[1] The phosphorylation states of proteins are strictly controlled by various protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). PTPs represent a diverse family of enzymes that exist as integral membrane and nonreceptor forms. Disorders of PTPs are likely to be related to several serious diseases such as cancers, autoimmune diseases, and diabetes, and the characterization of PTPs involved in such diseases is an important focus in biological research. Selective inhibitors of each PTPs are expected to be not only useful tools for clarifying the biological functions of the PTPs themselves but also candidates for novel therapeutics. In contrast to the findings of various specific inhibitors of protein kinases and protein serine/threonine phosphatases (PPs), until recently, only a limited number of compounds have been reported to be inhibitors of PTPs. Vanadate and phenyl arsine oxide (PAO) have been commonly used as PTP inhibitors in biological research; however, they inhibit almost all PTPs nonselectively only at relatively high concentrations and also inhibit other enzymes such as ATPase. As a result, they are highly toxic. Recent progress in biological research on PTPs has encouraged screening of PTP inhibitors, and several natural products and synthetic compounds, including many peptide or peptide-like compounds containing phosphonate or carboxylate, have been reported.[2,3] Most of these, however, likely have at least some problems regarding enzyme specificity, chemical and biological stability, and cell permeability.

Recently, among the PTPs, a group of enzymes that dephosphorylate both phosphotyrosine and phosphoserine/threonine have attracted increased attention. In particular, cdc25 phosphatases (cdc25A, -B, and -C) are considered to be important members of a family of dual-specificity phosphatases (DSPs) known to be key enzymes in cell-cycle progression. Cdc25 dephosphorylates and activates cyclin-dependent kinases. Several efforts to find effective cdc25 inhibitors have been reported. The first natural products reported to inhibit cdc25B were dnacin A1 and B1 (IC_{50} = 141 and 64.4 μM), but these compounds are also known to cause DNA damage.[4] Next, dysidiolide (IC_{50} = 9.4 μM)[5] and other synthetic carboxylic acid derivatives were reported to inhibit cdc25s with IC_{50} of micromolar orders.[6] The development of more potent inhibitors of cdc25 is still in great demand.

To find a selective inhibitor of cdc25 and also other biologically important PTPs/DSPs, we planned a focused library approach based on the structural feature of PTPs/DSPs. Catalytic sites of PTPs and DSPs share the highly conserved loop structure, $C(X)_5R$, which is thought to interact with the phosphate residue of phosphotyrosine. Figure 1 shows hydrogen bonds between the phosphate anion and NH groups of the amides in the loop structure, which was revealed by X-ray crystallography.[7] On the other hand, each enzyme has a unique structure surrounding the catalytic site that is expected to recognize amino acid residues of its specific substrate. The development of a library of compounds having a "core" structure that can interact with the conserved loop region instead of phosphate and having "various" substituents that may interact with the unique region

* To whom correspondence should be addressed. Address: Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba, Sendai 980-8577, Japan. Phone and fax: 81-22-217-5601. E-mail: sodeoka@tagen.tohoku.ac.jp.
† Tohoku University.
‡ Sagami Chemical Research Center.
§ RIKEN.

10.1021/jm0100741 CCC: $20.00
Published on Web 08/24/2001

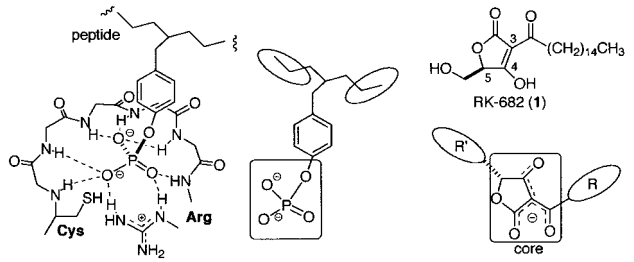


Figure 1.

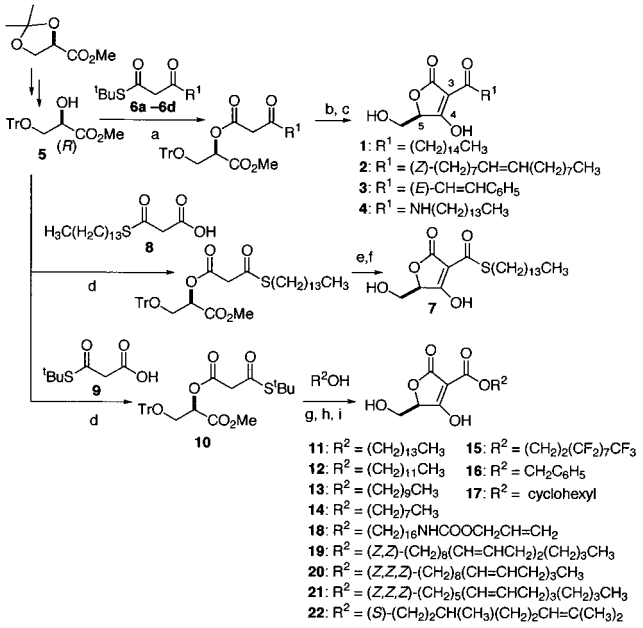
of each enzyme would be a good approach for identifying possible PTP/DSP selective inhibitors. A few library approaches to the identification of PTP inhibitors have been reported in which phosphonate or carboxylate seems to be used as "core" phosphate mimics.[8] Since phosphonate and carboxylate are known to interact with an active site of metallophosphatases, including PPs,[2b] we planned to find a novel "core" structure that can specifically interact with the active-site loop of PTP/DSP. Among the natural products reported to be inhibitors of PTPs, we picked up a tetronic acid derivative, RK-682 (**1**). RK-682 has been reported to inhibit a DSP, VHR (vaccinia VH1-related phosphatase), and a PTP, CD45 but not Ser/Thr protein phosphatase, PP1.[9] Since our kinetic analysis of VHR inhibition by RK-682 showed a competitive mode, the highly acidic 3-acyltetronic acid group could conceivably act as a phosphate mimic and might therefore be a candidate for the "core" structure that can distinguish the active-site structures of PTPs/DSPs from those of PPs. Accordingly, we decided to synthesize a library of tetronic acid derivatives with various substituents at C3 and C5 positions.

Here, we report the synthesis and structure−activity relationship (SAR) of the library of tetronic acid derivatives in relation to the inhibition of the dual-specificity phosphatases VHR[10] and cdc25B.[11]

Chemistry

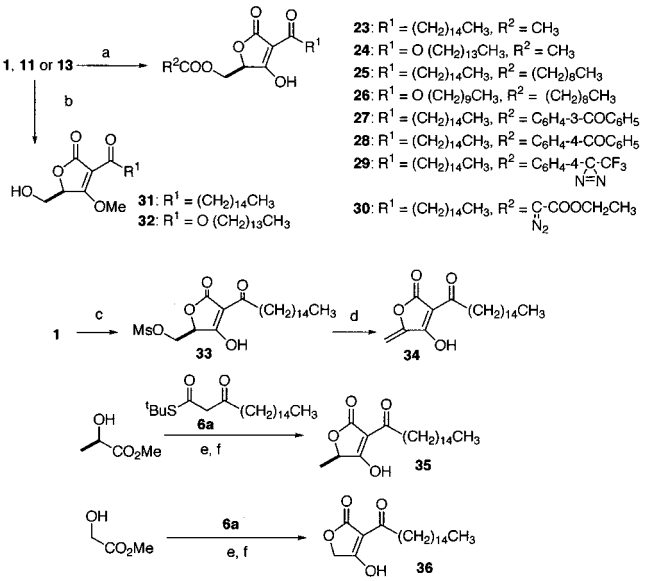
We have already reported an asymmetric synthesis of RK-682 (**1**), its enantiomer (ent-**1**), and its side chain analogues **2** and **3**, as well as the determination of their absolute stereochemistry.[12] Schemes 1 and 2 summarize the synthesis of various tetronic acid derivatives. 3-Carbamoyl derivative **4** was synthesized from **5** according to a procedure similar to that used in the synthesis of compounds **1**−**3**. More specifically, **6d** ($R^1 = NH(CH_2)_{13}CH_3$) was first prepared and then condensed with **5** by the treatment of silver trifluoroacetate. A tetronic acid ring was constructed by treatment with tetrabutylammonium fluoride (TBAF) and followed by deprotection of the trityl group, affording **4**. 3-Thiocarbonyl derivative **7** was prepared from the corresponding malonic acid half-thioester **8**. Furthermore, a wide variety of 3-alkoxycarbonyltetronic acid derivatives **11**−**22** were synthesized in good yields by the reaction of a common intermediate **10**, prepared from **5** and **9**, with the corresponding alcohols, followed by treatment of TBAF and HCl. 5-Acyloxymethyl derivatives **23**−**30** were prepared by the acylation of **1**, **11**, or **13**. 4-Methoxy derivatives **31** and **32** were obtained by the treatment of **1** and **11** with diazomethane. Mesylate **33** was also prepared, and subsequent elimination gave the exomethylene derivative **34**. 5-Methyl and 5-unsubstituted derivatives **35** and **36** were synthesized from methyl (*R*)-lactate and methyl glycolate, respectively, in a manner similar to the synthesis of **1**.

Scheme 1[a]



[a] (a) CF_3COOAg, THF, **6a** ($R^1 = (CH_2)_{14}CH_3$), **6b** ($R^1 = (Z)$-$(CH_2)_7CH{=}CH(CH_2)_7CH_3$), **6c** ($R^1 = (E)$-$CH{=}CHC_6H_5$), or **6d** ($R^1 = NH(CH_2)_{13}CH_3$). (b) Bu_4NF, THF. (c) 1 N aqueous HCl, MeOH (**1**, 75%; **2**, 64%; **3**, 43%; **4**, 61%, three steps from **5**). (d) DCC, CH_2Cl_2, **8** (67%) or **9** (**10**, 97%). (e) Bu_4NF, THF. (f) 1 N aqueous HCl, MeOH (**7**, 50%, two steps). (g) R^2OH, CF_3COOAg, THF. (h) Bu_4NF, THF. (i) 1 N aqueous HCl, MeOH (**11**, 63%; **12**, 28%; **13**, 58%; **14**, 58%; **15**, 40%; **16**, 38%; **17**, 51%; **18**, 57%; **19**, 71%; **20**, 64%; **21**, 52%; **22**, 78%, three steps from **10**).

Scheme 2[a]



[a] (a) Acylating reagent, pyridine, CH_2Cl_2 (Ac_2O; DMAP; **23**, 98%; **24**, 99%; $CH_3(CH_2)_8COCl$; **25**, 56%; **26**, 75%; 3-C_6H_5CO−C_6H_4COCl; **27**, 71%; 4-C_6H_5CO−C_6H_4COCl; **28**, 24%; 4-$CF_3C(N{=}N)$−C_6H_4COCl; **29**, 35%; $EtOCOCN_2COCl$; 2,6-lutidine; **30**, 60%). (b) CH_2N_2, THF (**31**, 56%; **32**, 93%). (c) MsCl, NEt_3, DMAP, CH_2Cl_2 (**33**, 64%). (d) 0.1 N NaOH, THF (**34**, 100%). (e) **6a**, CF_3COOAg, THF. (f) Bu_4NF, THF (**35**, 72%; **36**, 24%, two steps).

Table 1. Inhibition of VHR

compound	IC_{50} (μM)	compound	IC_{50} (μM)
1	11.6 ± 0.0	**21**	23.2 ± 0.6
ent-**1**	11.7 ± 0.4	**22**	>250
2	11.5 ± 0.5	**23**	12.8 ± 0.6
3	>100	**24**	11.1 ± 0.4
4	23.9 ± 1.0	**25**	37.9 ± 2.0
7	11.5 ± 0.4	**26**	37.2 ± 0.1
11	14.5 ± 0.6	**27**	4.0 ± 0.3
12	120 ± 0.0	**28**	4.6 ± 0.2
13	>250	**29**	4.7 ± 0.1
14	>250	**30**	12.4 ± 0.0
15	>250	**31**	>250
16	>250	**32**	>100
17	>250	**33**	9.2 ± 0.0
18	>250	**34**	9.5 ± 0.0
19	31.3 ± 0.3	**35**	10.6 ± 0.0
20	39.5 ± 0.9	**36**	14.4 ± 1.0

Results and Discussion

To evaluate our hypothesis that acyltetronic acid would bind to the active-site loop and that substituents at C3 and C5 would interact with the surrounding unique regions, we first examined the structure−activity relationships of these compounds as VHR inhibitors. Recently, VHR was found to dephosphorylate and inactivate ERKs, which are members of the MAPK family, and to play an important role in cell proliferation.[10c] Assays were performed using a recombinant VHR protein[13] and *p*-nitrophenyl phosphate (pNPP) as a substrate, and the results are summarized in Table 1. Conversion of the ketone at the C3 position in RK-682 to the ester (**11**) or thioester (**7**) did not affect the inhibition of VHR, but conversion to amide (**4**) slightly decreased inhibition. The amide group would restrict the conformational flexibility of the side chain and, as a result, could decrease the participation of the active conformer. Examination of a series of side chain analogues **11**−**22** indicated the importance of the hydrophobic side chain at the C3 position for the inhibition of VHR. Analogues with shorter side chain than C_{12} (**3**, **13**−**17**, and **22**), including those that were phenyl-, cyclohexyl-, or fluorine-substituted, showed negligible inhibition. The unsaturated C_{18} side chain analogues **19**−**21** exhibited lower levels of inhibitory activity than **11**. The introduction of an allyloxycarbonylamino group to the end of the C_{16} hydrophobic chain (**18**) drastically decreased the activity. Methylation of the enol group at the C4 position almost abolished the activity (**31** and **32**), emphasizing the importance of the free tetronic acid moiety. Modification of the C5-hydroxymethyl group affects the activity. Compounds having various substituted benzoyl groups (**27**−**29**) indicated significant increased inhibition of VHR compared to RK-682, whereas those having the long chain acyl group (**25** and **26**) exhibited lower levels of inhibition. The hydroxyl group at the C5 position itself does not seem to have any positive interaction with the enzyme because removal of the hydroxyl group did not affect activity (**34**−**36**). Furthermore, a preliminary molecular modeling study based on the reported crystal structure of VHR[14] was performed. These results supported our original hypothesis that the dissociated 3-acyltetronic acid anion can act as a "core" and tightly binds to the active-site loop by multiple hydrogen bondings (Figure 2). The hydrophobic side chain at the C3 position would be located in a relatively hydrophobic groove, and the 5-substituent can be oriented in the direction of the other possible recognition site for the substrate peptide. Inhibition of the Ser/Thr phosphatases, PP1, PP2A, and PP2C, was also tested for several compounds (**1**, ent-**1**, **2**, **3**, **7**, **12**−**15**, **19**−**22**, and **33**), and as expected, none of them showed significant inhibition up to 100 μM. These results indicated that the tetronic acid library is quite potent as a focused library oriented to the PTP/DSP-selective inhibitor.

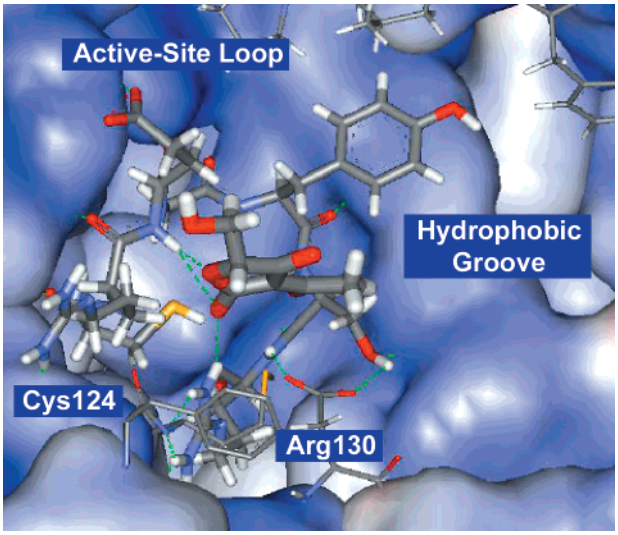


Figure 2. Model of 3-acetyl-5-hydroxymethyltetronic acid anion bound to VHR.

Table 2. Inhibition of Cdc25B

compound	IC_{50} (μM)	compound	IC_{50} (μM)
1	2.2 ± 0.0	**21**	1.6 ± 0.3
ent-**1**	5.1 ± 0.3	**22**	>125
2	0.90 ± 0.03	**23**	2.2 ± 0.1
3	32.1 ± 3.8	**24**	6.2 ± 0.2
4	7.9 ± 0.8	**25**	1.2 ± 0.2
7	3.6 ± 0.2	**26**	3.0 ± 0.2
11	18.0 ± 0.9	**27**	0.38 ± 0.02
12	11.5 ± 0.9	**28**	0.52 ± 0.03
13	35.0 ± 0.4	**29**	0.6 ± 0.0
14	100.8 ± 8.0	**30**	0.40 ± 0.0
15	26.2 ± 0.1	**31**	>125
16	>125	**32**	>125
17	>125	**33**	1.4 ± 0.1
18	22.9 ± 0.3	**34**	1.1 ± 0.0
19	2.0 ± 0.0	**35**	3.7 ± 0.3
20	3.3 ± 0.2	**36**	3.0 ± 0.1

We next examined the inhibition of cdc25B. Cdc25B is known to be a key enzyme for cell-cycle progression that selectively activates the cdc2−cyclin B complex by dephosphorylating both Tyr-15 and Thr-14 of cdc2, causing entry into the M phase.[10] Among the cdc25s, overexpression of cdc25B is known to be most well correlated to the malignancy of tumor cells, suggesting the importance of this enzyme in tumor progression. Thus, a specific inhibitor of cdc25B would be a candidate for a mechanistically distinct anticancer drug. Table 2 summarizes the IC_{50} values for the inhibition of cdc25B. Assays were performed using recombinant cdc25B and 3-*O*-methylfluorescein phosphate (OMFP) as a substrate. We were pleased to find that compounds **27**−**30** show quite a potent inhibition of cdc25B, with IC_{50} values of 0.38−0.6 μM.[15] It is also noteworthy that the following differences were observed in SAR for cdc25B and in that for VHR. (1) Long-chain hydrophobic sub-

Table 3. Inhibition of PTP-S2

compound	IC_{50} (μM)	compound	IC_{50} (μM)
1	>100	**21**	30.1 ± 1.1
ent-**1**	>100	**22**	>250
2	11.0 ± 0.4	**23**	>100
3	>100	**24**	>250
4	>100	**25**	>250
7	>100	**26**	>250
11	>50	**27**	>25
12	32.4 ± 4.9	**28**	>25
13	236.7 ± 7.0	**29**	>100
14	>180	**30**	>100
15	>250	**31**	>100
16	>250	**32**	>100
17	>250	**33**	>80
18	>250	**34**	>100
19	59.7 ± 18.0	**35**	>100
20	31.8 ± 8.4	**36**	>250

stituents at the C3 position are not as critical as for VHR (**1** vs **12**−**18**). (2) O-substitution at the α-position of the C3-acyl group is unfavorable for cdc25 compared to VHR (**1** vs **11**). (3) A long-chain hydrophobic substituent at the C5 position increased inhibition of cdc25 (**1** vs **25**), an effect opposite to that observed in the case of VHR. (4) Introduction of the diazomalonyl group improved inhibition of cdc25B, although no difference was observed in the inhibition of VHR (**1** vs **30**). As a result, compound **30** exhibited a strong inhibition of cdc25B (IC_{50} = 0.4 μM) and a 30-fold preference for cdc25B compared to VHR. To our knowledge, **27**−**30** are the most potent class of inhibitors among the reported cdc25 inhibitors.[6]

We also tested the inhibition of PTP-S2[16] as an example of tyrosine phosphatase. As shown in Table 3, the tetronic acid derivatives showed distinct profiles for the inhibition of PTP-S2. The potent cdc25B inhibitors **27**−**30** did not show any inhibition at the highest concentration used (depending on their solubility in assay solution), indicating high selectivity of these compounds. But it is noteworthy that some compounds such as **2** inhibited PTP-S2 with a reasonable IC_{50} value (11 μM).

Conclusions

A series of novel tetronic acid derivatives related to RK-682 were synthesized and evaluated as inhibitors of the dual-specificity protein phosphatases VHR and cdc25B. Despite only a limited type and number of compounds being synthesized and tested, several compounds were found to be stronger inhibitors of these DSPs than the parent natural product, RK-682. Compounds **27**−**30** inhibited cdc25B more potently than known inhibitors. Compound **30** also showed a 30-fold selectivity for cdc25B compared to the closely related DSP, VHR. Because of the ambiguous kinetics observed with cdc25B,[15] whether the tetronic acid "core" simply binds to the active site of this enzyme as we expected is not definitive. However, the promising results described herein strongly indicated that this tetronic acid library is quite potent as a library focused on the PTP/DSP-selective inhibitor. Expansion of the library and research to find more powerful and highly selective inhibitors of cdc25 and several other therapeutically important PTPs/DSPs are currently underway.

Experimental Section

General Methods. Infrared (IR) spectra were measured on a JASCO FT/IR-5300 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AM-400 and AC-200P or AVANCE 500 NMR spectrometer with tetramethylsilane used as an internal standard. Mass spectra (MS) were obtained with a Hitachi M-80B mass spectrometer using an electronic ionization method unless mentioned otherwise. Optical rotation was measured on a Horiba SEPA-200 polarimeter. In general, reactions were carried out under anhydrous conditions in dry solvents under argon atmosphere. The purity of the target compounds was determined to be more than 95% based on the ^{1}H NMR spectral analysis.

Synthesis of (*R*)-3-Hexadecanoyl-5-hydroxymethyltetronic Acid (1). According to the procedure described in the ref 12, **1** was synthesized from **5** and *S*-*tert*-butyl 3-oxooctadecanethioate (**6a**).

For preparation of **6b**−**6d**, see Supporting Information.

Synthesis of (*R*)-5-Hydroxymethyl-3-[(*Z*)-9-octadecenoyl]tetronic Acid (2). To a solution of *S*-*tert*-butyl (*Z*)-3-oxo-11-icosenethioate (**6b**) (712 mg, 1.79 mmol) and **5** (650 mg, 1.79 mmol) in THF (2.0 mL) was added silver trifluoroacetate (515 mg, 2.33 mmol), and the mixture was stirred at 23 °C for 5 h while being shielded from the light. The mixture was diluted with ether, passed through a short silica gel column, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt, 10:1) to give methyl (*R*)-2-[(*Z*)-3-oxo-11-icosenoyl]oxy-3-triphenylmethyloxypropionate (640 mg, 53%, a 5:1 mixture of keto and enol forms) as a colorless oil. To a solution of this ester (640 mg, 0.96 mmol) in THF (3.2 mL) was added tetrabutylammonium fluoride (TBAF, 1 M THF solution, 1.24 mL, 1.24 mmol), and the mixture was stirred at 23 °C for 2 h. After the addition of TBAF (0.19 mL, 0.19 mmol), the mixture was further stirred overnight at 23 °C. The reaction was quenched by the addition of 6 N aqueous HCl (0.26 mL, 1.57 mmol) and poured into ice−water; the mixture was then extracted with ether. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 1:0 to 20:1). The combined and concentrated fractions containing the desired product were redissolved in AcOEt (100 mL), washed with 0.5 N aqueous HCl and water, dried (Na_2SO_4), and concentrated to give (*R*)-3-[(*Z*)-9-octadecenoyl]-5-triphenylmethyloxymethyltetronic acid (563 mg, 92%) as a pale-yellow oil. To a solution of this compound (512 mg, 0.80 mmol) in methanol (50 mL) was added 1 N aqueous HCl (0.80 mL, 0.80 mmol), and the mixture was stirred at 23 °C for 88 h. After removal of the solvent in vacuo, the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 1:0 to 20:1 to 10:1). The combined and concentrated fractions containing the desired product were redissolved in AcOEt (100 mL), washed with 0.5 N aqueous HCl and water, dried (Na_2SO_4), and concentrated to give **2** (220 mg, 69%) as a colorless solid. The overall yield from **5** was improved to 64% (three steps) by omitting the column purification of methyl (*R*)-2-[(*Z*)-3-oxo-11-icosenoyl]oxy-3-triphenylmethyloxypropionate and (*R*)-3-[(*Z*)-9-octadecenoyl]-5-triphenylmethyloxymethyltetronic acid.

Synthesis of (*R*)-5-Hydroxymethyl-3-[(*E*)-3-phenyl-2-propenoyl]tetronic Acid (3) and (*R*)-5-Hydroxymethyl-3-tetradecylcarbamoyltetronic Acid (4). Similar to the synthesis of **2**, compounds **3** and **4** were synthesized by the reaction of **5** with *S*-*tert*-butyl (*E*)-3-oxo-5-phenyl-4-pentenethioate (**6c**) and *N*-tetradecyl-2-[(*tert*-butylthio)carbonyl]acetamide (**6d**), respectively (see Supporting Information).

Synthesis of (*R*)-5-Hydroxymethyl-3-(tetradecylthio)carbonyltetronic Acid (7). To a solution of **8** (234 mg, 0.74 mmol; for preparation, see Supporting Information) and **5** (206 mg, 0.57 mmol) in CH_2Cl_2 (1.5 mL) was added a solution of DCC (152 mg, 0.74 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C, and the mixture was stirred at 0 °C for 1.5 h. After removal of dicyclohexylurea by filtration, the filtrate was diluted with AcOEt, washed with 5% aqueous $NaHCO_3$, water, 1 N aqueous HCl, and water, dried (Na_2SO_4), and concentrated. The residue

was purified by silica gel column chromatography (hexane/AcOEt, 10:1) to give methyl (*R*)-2-[(tetradecylthio)carbonyl]-acetyloxy-3-triphenylmethyloxypropionate (326 mg, 67%) as a colorless oil. To a solution of this ester (326 mg, 0.49 mmol) in THF (1.6 mL) was added TBAF (1 M THF solution, 0.64 mL, 0.64 mmol), and the mixture was stirred at 23 °C for 64 h. During the reaction TBAF (0.10 mL after 2 h, 0.15 mL after 16 h, and 0.10 mL after 23 h) was further added to complete the reaction. The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 20:1) to give crude (*R*)-3-(tetradecylthio)carbonyl-5-triphenylmethyloxymethyltetronic acid (274 mg). This material was dissolved in methanol (26 mL), and 1 N aqueous HCl (0.44 mL) was added. After being stirred at 23 °C for 90 h, the reaction mixture was concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 20:1 to 10:1) and then treated with 0.5 N aqueous HCl to give **7** (94.6 mg, 50%) as a colorless solid.

Synthesis of Methyl (*R*)-2-[(*tert*-Butylthio)carbonyl]-acetyloxy-3-triphenylmethyloxypropionate (10). To a solution of **9** (119 mg, 0.67 mmol) and **5** (188 mg, 0.52 mmol) in CH_2Cl_2 (1.3 mL) was added a solution of DCC (139 mg, 0.67 mmol) in CH_2Cl_2 (0.5 mL) at 0 °C, and the mixture was stirred at 0 °C for 2 h. After removal of dicyclohexylurea by filtration, the filtrate was diluted with AcOEt, washed with 5% aqueous $NaHCO_3$, water, 0.5 N aqueous HCl, and water, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt, 10:1) to give **10** (262 mg, 97%) as a colorless oil.

Synthesis of (*R*)-5-Hydroxymethyl-3-tetradecyloxycarbonyltetronic Acid (11). To a solution of **10** (233 mg, 0.45 mmol) and tetradecanol (96 mg, 0.45 mmol) in THF (4.0 mL) was added silver trifluoroacetate (198 mg, 0.72 mmol), and the mixture was stirred for 5 h at 23 °C while shielded from light. The mixture was diluted with AcOEt, passed through a short silica gel column, and concentrated to give methyl (*R*)-2-(tetradecyloxycarbonyl)acetyloxy-3-triphenylmethyloxypropionate as a pale-yellow oil. To a solution of this ester (280 mg, 0.43 mmol) in THF (1.4 mL) was added TBAF (1 M THF solution, 0.56 mL, 0.56 mmol), and the mixture was stirred at 23 °C for 2 h. After addition of TBAF (0.09 mL) the mixture was further stirred at 23 °C for 6.5 h. The reaction was quenched by the addition of 4 N aqueous HCl and poured into ice−water; the mixture was then extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated to afford (*R*)-3-tetradecyloxycarbonyl-5-triphenylmethyloxymethyltetronic acid (475 mg) as a pale-yellow oil. To a solution of this crude product in methanol (45 mL) was added 1 N aqueous HCl (0.77 mL), and the mixture was stirred at 23 °C for 111 h. After removal of the solvent in vacuo, the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 1:0 to 20:1 to 10:1). The combined and concentrated fractions containing the desired product were redissolved in AcOEt (50 mL), washed with 0.5 N aqueous HCl and water, dried (Na_2SO_4), and concentrated to give **11** (105 mg, 63%) as a colorless solid.

Synthesis of 12−22. Similar to the synthesis of **11**, compounds **12**−**22** were synthesized from **10** and dodecanol (28%, colorless solid), decanol (58%, colorless solid), octanol (58%, colorless solid), 1*H*,1*H*,2*H*,2*H*-heptadecafluoro-1-decanol (40%, colorless solid), benzyl alcohol (38%, colorless solid), cyclohexyl alcohol (51%, pale-yellow amorphous solid), allyl (16-hydroxyhexadecyl)carbamate (57%, colorless solid), (9*Z*,12*Z*)-octadecadien-1-ol (71%, pale-yellow solid), (9*Z*,12*Z*,15*Z*)-octadecatrien-1-ol (64%, pale-yellow solid), (6*Z*,9*Z*,12*Z*)-octadecatrien-1-ol (52%, colorless solid), and (*S*)-*β*-citronellol (78%, colorless solid), respectively.

Synthesis of (*R*)-5-Acetoxymethyl-3-hexadecanoyltetronic Acid (23). To a solution of **1** (23.7 mg, 0.64 mmol) in CH_2Cl_2 (3 mL) was added acetic anhydride (0.017 mL, 0.18 mmol), pyridine (0.017 mL, 0.21 mmol), and a catalytic amount of DMAP, and the mixture was stirred at 23 °C for 28 h. The mixture was poured into ice−water and extracted with AcOEt. The organic layer was washed with 0.5 N aqueous HCl, water, saturated aqueous $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 10:1) to give **23** (25.8 mg, 98%) as a colorless solid.

Synthesis of (*R*)-5-Acetoxymethyl-3-tetradecyloxycarbonyltetronic Acid (24). Similar to the synthesis of **23**, compound **24** was synthesized from **11** (99%, colorless solid).

Synthesis of (*R*)-5-Decanoyloxymethyl-3-hexadecanoyltetronic Acid (25). To a solution of **1** (15.5 mg, 0.042 mmol) in CH_2Cl_2 (0.175 mL) was added decanoyl chloride (0.017 mL, 0.084 mmol) and pyridine (0.007 mL, 0.087 mmol) at 0 °C, and the mixture was stirred at 23 °C for 26 h. To this mixture saturated aqueous $NaHCO_3$ was added at 0 °C, and the mixture was stirred at 23 °C for 20 min. The mixture was acidified with 1 N aqueous HCl and extracted with CH_2Cl_2. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 50:1 to 20:1) to give **25** (12.4 mg, 56%) as a pale-yellow solid.

Synthesis of (*R*)-5-Decanoyloxymethyl-3-tetradecyloxycarbonyltetronic Acid (26). Similar to the synthesis of **25**, compound **26** was synthesized from **11** (75%, colorless solid).

Synthesis of (*R*)-5-(3-Benzoylbenzoyl)oxymethyl-3-hexadecanoyltetronic Acid (27). To a solution of **1** (19.3 mg, 0.052 mmol) in CH_2Cl_2 (0.40 mL) was added a solution of 3-benzoylbenzoyl chloride (19.4 mg, 0.079 mmol) in CH_2Cl_2 (0.15 mL) and pyridine (0.007 mL, 0.087 mmol) at 0 °C, and the mixture was stirred at 23 °C for 3 h. After addition of a solution of 3-benzoylbenzoic acid chloride (12.8 mg, 0.052 mmol) in CH_2Cl_2 (0.15 mL) and pyridine (0.004 mL, 0.049 mmol) at 0 °C, the mixture was further stirred at 23 °C for 1 h. The reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ at 0 °C, stirred at 23 °C for 20 min, acidified with 1 N aqueous HCl, and extracted with CH_2Cl_2. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 1:0 to 10:1) and then treated with 0.5 N HCl to give **27** (21.3 mg, 71%) as a pale-yellow solid.

Synthesis of (*R*)-5-(4-Benzoylbenzoyl)oxymethyl-3-hexadecanoyltetronic Acid (28). Similar to the synthesis of **27**, compounds **28**−**30** were synthesized from **1** and 4-benzoylbenzoyl chloride (24%, pale-yellow solid), 4-(1-azi-2,2,2-trifluoroethyl)benzoyl chloride (35%, pale-yellow solid), and ethoxycarbonyldiazoacetyl chloride (60%, pale-yellow amorphous solid) (see Supporting Information).

Synthesis of (*R*)-3-Hexadecanoyl-5-hydroxymethyl-4-methoxy-furan-2-(5*H*)-one (31). To a solution of **1** (30.0 mg, 0.081 mmol) in THF (0.5 mL) was added a solution of excess diazomethane in Et_2O at 23 °C, and the mixture was stirred at 23 °C for 20 min. After addition of a small amount of acetic acid to quench the excess diazomethane, the mixture was diluted with AcOEt, washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 50:1) and then treated with 0.5 N HCl to give **31** (17.5 mg, 56%) as a colorless amorphous solid.

Synthesis of (*R*)-5-Hydroxymethyl-4-methoxy-3-tetradecyloxycarbonyl-furan-2-(5*H*)-one (32). Similar to the synthesis of **31**, compound **32** was synthesized from **11** (93%, colorless amorphous solid).

Synthesis of (*R*)-3-Hexadecanoyl-5-methanesulfonyloxymethyltetronic Acid (33) and 3-Hexadecanoyl-5-methylenetetronic Acid (34). To a solution of **1** (50 mg, 0.14 mmol) in THF (0.6 mL) were added methanesulfonyl chloride (0.021 mL, 0.27 mmol), triethylamine (0.076 mL, 0.54 mmol), and DMAP (8 mg, 0.07 mmol) at 23 °C, and the mixture was stirred at 23 °C for 5 h. The mixture was poured into ice−water and extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 10:1) and then treated with 0.5 N HCl to give **33** (38 mg, 64%) as a pale-yellow solid. To a solution of **33** (13 mg, 0.03 mmol) in THF (2.0 mL) was added 0.1 N aqueous

NaOH (1.0 mL), and the mixture was stirred at 23 °C for 69 h. The mixture was acidified with 1 N HCl to pH 1−2 and extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 10:1) and then treated with 0.5 N HCl to give **34** (14 mg, 100%) as a colorless solid.

Synthesis of (*R*)-3-Hexadecanoyl-5-methyltetronic Acid (35). To a solution of **6a** (400 mg, 1.08 mmol) and methyl (*R*)-lactate (0.10 mL, 1.08 mmol) in THF (7.0 mL) was added silver trifluoroacetate (240 mg, 1.08 mmol), and the mixture was stirred overnight at 23 °C while shielded from light. After addition of silver trifluoroacetate (100 mg, 0.45 mmol), the mixture was stirred for 2 days at 23 °C. The mixture was quenched by the addition of water, extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt, 10:1) to give methyl (*R*)-2-(3-oxo-octadecanoyl)oxypropionate (369 mg, 89%, a 2:1 mixture of keto and enol forms) as a colorless oil. To a solution of this ester (224 mg, 0.58 mmol) in THF (1.0 mL) was added TBAF (1 M THF solution, 0.87 mL, 0.87 mmol), and the mixture was stirred at 23 °C for 1 day. After addition of TBAF (0.87 mL, 0.87 mmol), the mixture was further stirred overnight at 23 °C. The reaction was quenched by the addition of 1 N aqueous HCl (100 mL), and the mixture was then extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 30:1) and thin layer silica gel chromatography and then was treated with 0.5 N aqueous HCl to give **35** (166 mg, 81%) as a pale-yellow solid.

Synthesis of 3-Hexadecanoyltetronic Acid (36). Similar to the synthesis of **35**, compound **36** was synthesized from **6a** and methyl glycolate (24%, pale-yellow solid; see Supporting Information).

VHR Assays. VHR was overexpressed in *E. coli* and purified. Assays were performed using pNPP as a substrate at 37 °C in 50 mM succinate, 1 mM EDTA, 150 mM NaCl, pH 6.0, in the presence or absence of the compound. Immediately after addition of purified VHR (0.6 μM) and pNPP (10 mM), the reaction mixture (200 μL) was incubated at 37 °C for 3 min. The phosphatase reaction was then terminated by the addition of 1 N NaOH (1 mL). The phosphatase activities were measured as absorbance changes at 405 nm.

Cdc25B Assays. Cdc25B (aa 377−565) was overexpressed as His_6-tag-fusion protein in *E. coli* and purified. Assays were performed using OMFP as a substrate at 37 °C in 100 mM Tris, 1 mM DTT, 40 mM NaCl, 20% glycerol, pH 8.2, in the presence or absence of the compound. Immediately after the addition of purified cdc25B (0.04 μM) and OMFP (200 μM), the reaction mixture (700 μL) was incubated at 37 °C for 20 min. The phosphatase reaction was then terminated by the addition of 1 M MES (pH 5.5)−6 M guanidine (150 μL). The phosphatase activities were measured as absorbance changes at 477 nm.

Molecular Modeling. The initial structure generated by docking 3-acetyltetronic acid anion to VHR manually was optimized by molecular mechanics calculation using the computer program Hyper-Chem (Hypercube Inc., Canada). Further analysis of the 3D structure of the VHR−RK682 (**1**) complex using molecular dynamics simulation will be published elsewhere.

Acknowledgment. We thank Professor Hiroto Okayama (University of Tokyo) for the generous gift of cdc25 cDNA. This work was supported in part by The Hayashi Memorial Foundation for Female Natural Scientists, The Kato Memorial Bioscience Foundation, and The Mochida Memorial Foundation for Medical and Pharmaceutical Research.

Supporting Information Available: Spectral data for compounds **2**−**36**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Fischer, E. H.; Charbonneau, H.; Tonks, N. K. Protein Tyrosine Phosphatases: A Diverse Family of Intracellular and Transmembrane Enzymes. *Science* **1991**, *253*, 401−406. (b) Stone, R. L.; Dixon, J. E. Protein-tyrosine Phosphatases. *J. Biol. Chem.* **1994**, *269*, 31323−31326. (c) Sun, H.; Tonks, N. K. The coordinated action of protein tyrosine phosphatases and kinases in cell signaling. *Trends Biochem. Sci.* **1994**, *19*, 480−485 and references therein.

(2) (a) Burke, T. R., Jr.; Zhang, Z.-Y. Protein-Tyrosine Phosphatases: Structure, Mechanism, and Inhibitor Discovery. *Biopolymer* **1998**, *47*, 225−241. (b) Widlanski, T. S.; Myers, J. K.; Stec, B.; Holtz, K. M.; Kantrowitz, E. R. The road less travelled: taming phosphatases. *Chem. Biol.* **1997**, *4*, 489−492 and references therein.

(3) Last year, non-peptide-like reversible inhibitors of PTP1B were reported. (a) Andersen, H. S.; Iversen, L. F.; Jeppesen, C. B.; Branner, S.; Norris, K.; Rasmussen, H. B.; Møller, K. B.; Møllere, N. P. H. 2-(Oxalylamino)-benzoic Acid Is a General, Competitive Inhibitor of Protein-tyrosine Phosphatases. *J. Biol. Chem.* **2000**, *275*, 7101−7108. (b) Malamas, M. S.; Sredy, J.; Moxham, C.; Katz, A.; Xu, W.; McDevitt, R.; Adebayo, F. O.; Sawicki, D. R.; Seestaller, L.; Sullivan, D.; Taylor, J. R. Novel Benzofuran and Benzothiophene Biphenyls as Inhibitors of Protein Tyrosine Phosphatase 1B with Antihyperglycemic Properties. *J. Med. Chem.* **2000**, *43*, 1293−1310.

(4) Horiguchi, T.; Nishi, K.; Hakoda, S.; Tanida, S.; Nagata, A.; Okayama, H. Dnacin A1 and dnacin B1 are antitumor antibiotics that inhibit cdc25B phosphatase activity. *Biochem. Pharmacol.* **1994**, *48*, 2139−2141.

(5) (a) Gunasekera, S. P.; McCathy, P. J.; Kelly-Borges, M.; Lobkovsky, E.; Clardy, J. Dysidiolide: A Novel Protein Phosphatase Inhibitor from the Caribbean Sponge *Dysidea etheria* de Laubengels. *J. Am. Chem. Soc.* **1996**, *118*, 8759−8760. (b) Blanchard, J. L.; Epstein, D. M.; Boisclair, M. D.; Rudolph, J.; Pal, K. Dysidiolide and Related γ-Hydroxy Butenolide Compounds as Inhibitors of the Protein Tyrosine Phosphatase, CDC25. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2537−2538. (c) Takahashi, M.; Dodo, K.; Sugimoto, Y.; Aoyagi, Y.; Yamada, Y.; Hashimoto, Y.; Shirai, R. Synthesis of the Novel Analogues of Dysidiolide and Their Structure−Activity Relationship. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2571−2574.

(6) (a) Rice, R. L.; Rusnak, J. M.; Yokokawa, F.; Yokokawa, S.; Messner, D. J.; Boynton, A. L.; Wipf, P.; Lazo, J. S. A Targeted Library of Small-Molecule, Tyrosine, and Dual-Specificity Phosphatase Inhibitors Derived from Rational Core Design and Random Side Chain Variation. *Biochemistry* **1997**, 15965−15974. (b) Ham, S. W.; Park, H. J.; Lim, D. H. Studies on Menadione as an Inhibitor of the cdc25 Phosphatase. *Bioorg. Chem.* **1997**, *25*, 33−36. (c) Peng, H.; Zalkow, L. H.; Abraham, R. T.; Powis, G. Novel CDC25A Phosphatase Inhibitors from Pyrolysis of 3-α-Azido-B-homo-6-oxa-4-cholesten-7-one on Silica Gel. *J. Med. Chem.* **1998**, *41*, 4677−4680. (d) Bergnes, G.; Gilliam, C. L.; Boisclair, M. D.; Blanchard, J. L.; Blake, K. V.; Epstein, D. M.; Pal, K. Generation of an Ugi Library of Phosphate Mimic-Containing Compounds and Identification of Novel Dual Specific Phosphatase Inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2849−2854. (e) El-Subbagh, H. I.; Abadi, A. H.; Al-Khawad, I. E.; Al-Rashood, K. A. Synthesis and Antitumor Activity of Some New Substituted Quinolin-4-one and 1,7-Naphthyridin-4-one Analogs. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 19−24. (f) Dodo, K.; Takahashi, M.; Yamada, Y.; Sugimoto, Y.; Hashimoto, Y.; Shirai, R. Synthesis of a Novel Class of cdc25A Inhibitors from Vitamin D_3. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 615−617. (g) Wipf, P.; Aslan, D. C.; Luci, D. K.; Southwick, E. C. Synthesis and Biological Evaluation of a Targeted Library of Protein Phosphatase Inhibitors. *Biotech. Bioeng.* **2000**, *71*, 58−70 and references therein.

(7) Jia, Z.; Barford, D.; Flint, A. J.; Tonks, N. K. Structural Basis for Phosphotyrosine Peptide Recognition by Protein Tyrosine Phosphatase 1B. *Science* **1995**, *268*, 1754−1758.

(8) (a) Moran, E. J.; Sarshar, S.; Cargill, J. F.; Shahbaz, M. M.; Lio, A.; Mjalli, A. M. M.; Armstrong, R. W. Radio Frequency Tag Encoded Combinatorial Library Method for the Discovery of Tripeptide-Substituted Cinnamic Acid Inhibitors of the Protein Tyrosine Phosphatase PTP1B. *J. Am. Chem. Soc.* **1995**, *117*, 10787−10788. (b) Cao, X.; Moran, E. J.; Siev, D.; Lio A.; Ohashi, C.; Mjalli, A. M. M. Synthesis of NH−Acyl-α-aminoamides on Rink Resin: Inhibitors of the Hematopoietic Protein Tyrosine Phosphatase (HePTP). *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2953−2958 and references therein. See also refs 6a, 6d, and 6g.

(9) Hamaguchi, T.; Sudo, T.; Osada, H. RK-682, a potent inhibitor of tyrosine phosphatase, arrested the mammalian cell cycle progression at G_1 phase. *FEBS Lett.* **1995**, *372*, 54−58.

(10) (a) Ishibashi, T.; Bottaro, D. P.; Chan, A.; Miki, T.; Aaronson, S. A. Expression cloning of a human dual-specificity phosphatase. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 12170−12174. (b) Aroca, P.; Bottaro, D. P.; Ishibashi, T.; Aaronson, S. A.;

Santos, E. Human Dual Specificity Phosphatase VHR Activates Maturation Promotion Factor and Triggers Meiotic Maturation in *Xenopus* Oocytes. *J. Biol. Chem.* **1995**, *270*, 14229−14234. (c) Todd, J. L.; Tanner, K. G.; Denu, J. M. Extracellular Regulated Kinases (ERK)1 and ERK2 Are Authentic Substrates for the Dual-Specificity Protein-Tyrosine Phosphatase VHR. *J. Biol. Chem.* **1999**, *274*, 13271−13280.

(11) (a) Nagata, A.; Igarashi, M.; Jinno, S.; Suto, K.; Okayama, H. An Additional Homolog of the Fission Yeast *cdc25*$^{+}$ Gene Occurs in Humans and Is Highly Expressed in Some Cancer Cells. *New Biol.* **1991**, *3*, 959−968. (b) Galaktionov, K.; Beach, D. Specific Activation of cdc25 Tyrosine Phosphatases by B-Type Cyclins: Evidence for Multiple Roles by Mitotic Cyclins. *Cell* **1991**, *67*, 1181−1194. (c) Sadhu, K.; Reed, S. I.; Richardson, H.; Russell, P. Human homolog of fission yeast cdc25 mitotic inducer is predominantly expressed in G_2. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5139−5143. (d) Honda, R.; Ohba, Y.; Nagata, A.; Okayama, H.; Yasuda, H. Dephosphorylation of human $p34^{cdc2}$ kinase on both Thr-14 and Tyr-15 by human cdc25B phosphatase. *FEBS Lett.* **1993**, *318*, 331−334. (e) Hoffman, I.; Draetta, G.; Karsenti, E. Activation of the phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G_1/S transition. *EMBO J.* **1994**, *13*, 4302−4310.

(12) (a) Sodeoka, M.; Sampe, R.; Kagamizono, T.; Osada, H. Asymmetric Synthesis of RK-682 and Its Analogs, and Evaluation of Their Protein Phosphatase Inhibitory Activity. *Tetrahedron Lett.* **1996**, *37*, 8775−8778. (b) Sodeoka, M.; Sampe, R.; Kojima, S.; Baba, Y.; Morisaki, N.; Hashimoto, Y. Asymmetric Synthesis of a 3-Acyltetronic Acid Derivative, RK-682, and Formation of Its Calcium Salt during Silica Gel Column Chromatography. *Chem. Pharm. Bull.* **2001**, *49*, 206−212.

(13) Usui, T.; Marriott, G.; Inagaki, M.; Swarup, G.; Osada, H. Protein phosphatase 2A inhibitors, phoslactomycins. Effects on the cytoskeleton in NIH/3T3 cells. *J. Biochem.* **1999**, *125*, 960−965.

(14) Yuvaniyama, J.; Denu, J. M.; Dixon, J. E.; Saper, M. A. Crystal Structure of the Dual Specificity Protein Phosphatase VHR. *Science* **1996**, *272*, 1328−1331.

(15) Preliminary kinetic analysis suggested that **30** inhibits cdc25B in a mixed-type manner.

(16) Reddy, R. S.; Swarup, G. Alternative splicing generates four different forms of a non-transmembrane protein tyrosine phosphatase mRNA. *DNA Cell Biol.* **1995**, *14*, 1007−1015. For expression and assay of PTP-S2, see ref 13.

JM0100741